



Patent  
Attorney's Docket No. 000475-205

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of )  
Daniel Jed Harrison, et al. )  
Application No.: 08/858,087 ) Group Art Unit: 1641  
Filed: May 16, 1997 ) Examiner: V. Portner  
For: MICROFLUIDIC SYSTEM AND )  
METHODS OF USE )

**DECLARATION PURSUANT TO 37 C.F.R. § 1.131**

Assistant Commissioner for Patents  
Washington, D.C. 20231

We, DANIEL JED HARRISON, PER ANDERSSON, PAUL C.H. LI, RODERICK SZARKA and RICHARD SMITH hereby declare that:

1. We are the joint inventors of the subject matter of the now presented claims in the above-captioned application.

2. Prior to August 2, 1996, we were collaborating on research conducted in Canada relating to a method of observing the effect of one or more candidate compounds on cells in a microfluidic device comprising:

- (a) providing a microfluidic device comprising a main flow path comprising a detection zone, and an outlet and at least two inlet flow paths intersecting and merging with the main flow path at or upstream of the detection zone;
- (b) applying at least one cell which is capable of flowing in the microfluidic device to a first inlet flow path and a candidate compound to a second inlet flow path;
- (c) inducing flow of the cells and the candidate compound toward the outlet;
- (d) allowing the cells to mix with the candidate compound; and
- (f) observing the effect of the candidate compound on the cells in the detection zone.

4. In Exhibit A, all non-pertinent material has been blacked out and the dates

on Exhibit A which are prior to August 2, 1996 have been removed.

5. Prior to August 2, 1996 we developed a method of observing the effects of one or more candidate compounds on cells in a microfluidic device by

- (a) providing a microfluidic device comprising a main flow path comprising a detection zone, and an outlet and at least two inlet flow paths intersecting and merging with the main flow path at or upstream of the detection zone;
- (b) applying at least one cell which is capable of flowing in the microfluidic device to a first inlet flow path and a candidate compound to a second inlet flow path;
- (c) inducing flow of the cells and the candidate compound toward the outlet;
- (d) allowing the cells to mix with the candidate compound; and
- (f) observing the effect of the candidate compound on the cells in the detection zone.

6. Attached hereto as Exhibit A are lab notebook pages for the experiments discussed below, all of which were completed prior to August 2, 1996.

7. Prior to August 2, 1996, the idea of retaining cells in a reaction chamber was conceived. Exhibit A, pages 1, 2, 8 and 8A evidence the idea of using baffles or weirs to define the chamber in the microchip, the idea of using fluid flow resistance to control the flow and the layout and dimensions of the microchip channels.

8. Prior to August 2, 1996, glass devices were fabricated at the Alberta Microelectronic Centre, using a modified silicon micromachining technique, for working with cells.

9. Prior to August 2, 1996, the electrophoretic mobility of red blood cells and yeast cells were tested in a microchip as demonstrated in Exhibit A, pages 10- 11. Specifically, pages 10 and 11 of Exhibit A evidences that a phosphate buffer of  $\text{Na}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$  was used as an isotonic buffer for canine erythrocytes. The canine erythrocytes were moved through the microchip using an electric field. Pages 10 and 11 of Exhibit A also evidence an experiment which showed that yeast cells flowed through the

microchip in response to an electric field.

10. Prior to August 2, 1996, the electrophoretic mobility of red blood cells, was tested. Specifically, pages 19 to 20 of Exhibit A demonstrate canine red blood cells were mobilized on a PCRD2 microchip. The blood sample was centrifuged for separation into various blood components. After removal of the plasma the erythrocytes were isolated as a cell pellet. A citrate-phosphate-dextrose (CPD) solution was used. The cells were washed several times with the isotonic phosphate buffer to remove the storage buffer. The layout of the PCRD2 device used for this work is shown in Exhibit B, Figure 1B which corresponds to Figure 5B in the application. The cells were loaded into the device by pressure. Potentials were applied to the various reservoirs to direct cell flow within the four intersecting channels.

Pages 20 to 22 of Exhibit A evidence an experiment involving the lysis of erythrocytes (or hemolysis) by a detergent in the COPI microchip. The layout of the COPI device is shown in Exhibit B, Figure 1A which corresponds to Figure 5A in the application. The cells were loaded onto the device by applying pressure and electric potentials were then applied to the various reservoirs to direct cell flow. Pressure was applied to deliver the cells. The reaction occurred after mixing a stream of cells at an intersection with another stream containing a lysing agent. It was found that the anionic detergent, sodium dodecyl sulfate (SDS), lysed cells sufficiently rapidly that the lysing process could be followed in a flowing stream within the chip.

11. Prior to August 2, 1996, three cell types, red blood cells, yeast cells and *E. coli* cells were mobilized by an electric field on the microchip. Pages 35A and 35 B of Exhibit A provide evidence of the manipulation of the three cell types on a chip.

The experiment for yeast cell transport was performed with the COPI device, illustrated on page 35A. During loading of yeast cells, the sample reservoir (S) was at +100 V, while the sample waste (SW) reservoir was at ground. During injection, the buffer reservoir (B) was set at +500 V.

The experiment for *E. coli* cell transport was performed at the Y-intersection of the PCRD2 device, illustrated in Exhibit B, Figure 1B. The cells were introduced at the BW1 reservoir whose potential is at ground. The BW2 reservoir was at -200 V. The potentials of the other reservoirs (B, S, and SW) were floating initially. To control flow direction, -200 V was then toggled back and forth between BW2 and SW reservoirs, leaving the alternate reservoir floating.

12. Prior to August 2, 1996 the use of calcium indicator dyes such as Fluo-3 AM to observe calcium influx was considered as was the use of activators such as concanavalin A (Con A) and the calcium ionophore calcimycin (A23187). Exhibit A, pages 36 - 38 evidence this concept.

13. Prior to August 2, 1996, Con A was used to activate or stain yeast cells in a microchip device. Pages 52 - 58 of Exhibit A evidence that the cells were observed in the COPI device. The reaction occurred at the point of mixing. Either pressure or electric fields were used to move the reagents in the microchip device. An Olympus microscope, at 20X magnification, dark field was used to observe the cell reactions.

14. Prior to August 2, 1996, the measurement of calcium ion influx into rat lymphocytes was induced by an ionophore calcimycin (A23187, calcium ionophore) as evidenced by pages 59 to 63 of Exhibit A. For measurement of calcium ion influx, a calcium-specific fluorophore, Fluo-3 AM was preloaded into purified rat lymphocytes as an acetoxyethyl (AM) ester. A sample of Fluo-3 AM ester solution was diluted into HEPES buffer. The lymphocyte suspension was mixed with the Fluo-3 AM ester solution to make a lymphocyte-Fluo-3 AM labeling solution. After lymphocyte labeling, the cells were washed with HEPES buffer. The labeled lymphocytes were suspended in HEPES buffer. The lymphocytes were loaded into the PCRD2 device. The A23187 was loaded into the microchip device and the cells were visually observed. The use of an inhibitor with an activator was considered, as evidenced on page 59 of Exhibit A where the use of theophylline with the activator Con A is described.

15. Prior to August 2, 1996 and as evidenced by pages 64 to 70 of Exhibit A, the measurement of calcium ion influx into mouse lymphocytes was induced by a calcium releasing agent calcimycin (A23187, calcium ionophore). A sample of Fluo-3 AM solution was diluted into HEPES buffer. The lymphocyte cell solution was mixed with the Fluo-3 AM solution to make a lymphocyte-Fluo-3 AM labeling solution. After lymphocyte labeling, the cells were washed with HEPES buffer. The labeled lymphocytes were suspended in HEPES buffer. The lymphocytes were loaded into the PCRD2 device. The A23187 was loaded into the microchip device and the cells were observed. A fluorescence microscope and a CCD camera was positioned over the detection zone and allowed the observation of cells as they passed the detection zone. The device was operated in a continuous flow mode. At the mixing point, the lymphocyte showed fluorescence caused by calcium influx upon activation with A23187 and the progress of the cells was monitored by following the cells down the stream.

16. Prior to August 2, 1996 and as evidenced by pages 79 - 81, 95 and 96 of Exhibit A, the weir device was designed. Two weir devices were used in the microfluidic device. The distance between the weirs in the first device was 1  $\mu\text{m}$  and in the second device it was 3  $\mu\text{m}$ . Tests of the weir devices with yeast cells showed that the cells could be trapped or stopped while the fluid flowed past the weirs.

17. In view of all of the facts set forth above and prior to August 2, 1996, it was concluded that it was possible to observe the effects of a candidate compound on cells in a microfluidic device comprising a main flow path comprising a detection zone, and an outlet and at least two inlet flow paths intersecting and merging with the main flow path at or upstream of the detection zone by applying at least one cell which flows in the microfluidic device to a first inlet flow path and a candidate compound to a second inlet flow path and inducing flow of the cells and the candidate compound toward the outlet, allowing the cells to mix with the candidate compound and observing the effect of the candidate compound on the cells in the detection zone.

The undersigned joint inventors declare further that all statements made herein of

their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at \_\_\_\_\_, the \_\_\_ day of August, 1999

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Daniel Jed Harrison

Signed at \_\_\_\_\_, the \_\_\_ day of August, 1999

\_\_\_\_\_  
Per Andersson

Signed at Burnaby BC, the 16 day of August, 1999

  
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Paul C.H. Li

Signed at \_\_\_\_\_, the \_\_\_ day of August, 1999

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Roderick Szarka

Signed at \_\_\_\_\_, the \_\_\_ day of August, 1999

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Richard Smith